Trends in Microbiology

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Review

Phage tRNAs: decoding the enigma

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The presence of tRNAs in bacteriophage genomes has intrigued scientists since their discovery in the early 1960s, as phages were believed to rely on the host tRNAs for their translation. Over the years, a multitude of hypotheses have been explored, providing evidence that phages with different lifestyles utilize tRNAs in distinct ways. In recent years, several studies have provided evidence that phage tRNAs play a crucial role in evading phage defense systems. In this review we summarize the current state of the field of phage tRNAs, highlighting their diverse roles in phage infection. We also discuss the host response to phage tRNAs and the application of this knowledge to improve phage-based therapeutics to combat bacterial infections.

Highlights

The existence of tRNAs in phage genomes has intrigued scientists since their discovery in the early 1960s.

Over the years, phage tRNAs were found to serve a multitude of functions, including compensating for their integration in the host tRNA, broadening the host range, and timing their lytic cycle.

Recent breakthroughs have also shown that phage tRNAs counteract the tRNA-depleting host response upon detecting a phage infection.

These phage tRNAs are cleavage-resistant and have potential to improve phage-therapeutic approaches by rendering these defense responses ineffective.

Viruses of bacteria encode transfer RNAs for translation

Transfer RNAs (tRNAs) were first discovered in the 1950s [1] and have since been recognized to be vital in the central dogma of molecular biology in all living systems (Box 1) [2]. Surprisingly, during the 1960s, tRNAs were also reported in the viruses of bacteria (phages) [3]. This finding challenged the view of viruses at that time, which were believed to hijack the host translation machinery for their replication, including the host tRNAs instead of encoding their own [4]. Follow-up studies observed that the tRNAs from *Escherichia coli* phage T4 were expressed early in the infection and that removing these tRNAs from the genome resulted in a 20-fold reduced infectivity [5,6]. Intriguingly, recent efforts found that phages more often encode tRNAs than previously thought, observing tRNAs in more than 30% of phage genomes, ranging from 1 to 62 tRNAs [7]. Yet, despite their widespread occurrence, the precise function of phage tRNAs during the **infection cycle** (see Glossary) remains unclear. Recent breakthroughs have uncovered unexpected roles for these tRNAs, from circumventing the immune response of the host to regulating the phage replication cycle. We summarize these recent advances in this review.

The diverse roles of phage tRNAs

The role of tRNA genes in phage genomes has been extensively discussed and explored, with numerous studies identifying a diverse set of potential roles that can be attributed to phage tRNAs [20,21]. One important factor in this discussion is that the lifestyle of the tRNA-encoding phage appears to determine the specific function of the tRNA. These lifestyles can be grouped into two main types: **virulent phages** and **temperate phages** [22]. Virulent phages follow the **lytic cycle**, in which phage replication and formation of new particles start upon genome ejection into the host cell, while temperate phages can follow the **lysogenic cycle**, in which they integrate into the genome of the host. Here, the temperate phage can remain integrated, passively replicating itself within the host chromosome. Under stressful conditions for the host, temperate phages excise from the host genome and transition to the lytic cycle, like virulent phages, to abandon ship. Some types of temperate phages, such as plasmid-phages, deviate from the lysogenic cycle by not integrating in the chromosome, but instead, these phages replicate as plasmids [23]. Interestingly, temperate phages typically encode just a few phage tRNAs, while virulent phages are found to encode a multitude, sometimes up to 61 different tRNAs [20,24]. This suggests that phages with different life cycles

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may have different needs for tRNAs. Over the years, several hypotheses and roles have been proposed for phage tRNAs.

Diverse functions of tRNA from temperate phages

As mentioned earlier, for temperate phages that integrate into the tRNA gene of their hosts, the encoded phage tRNAs may replace the function of the dysfunctional host tRNA that the temperate phage integrates into during its lysogenic cycle. In the majority of observed cases, host tRNA genes are a prime integration target of temperate phages, since host tRNAs are prevalent and conserved across a large diversity of hosts, making it a reliable integration site [25,26]. A disadvantage of integrating into the host tRNAs is that the function of this host tRNA is disrupted, leading to a disadvantage for the host, and consequently, for the integrated phage that relies on host survival for its own existence. To resolve this downside, some phages encode the same tRNA in their genome to replace the function of the integrated host tRNA (Figure 1A) [20,27]. Alternatively, some temperate phages, such as Mlo38S, are flanked by tRNA halves at the 3′ and 5′ end of their genome, which forms two complete functional tRNA copies when it integrates into the tRNA of the host (Figure 1B) [28,29]. Notably, temperate phages often encode multiple tRNAs, suggesting that the function of phage tRNAs in temperate phages is not limited to compensating for the disrupted tRNA of the host.

Several hypotheses could explain these additional tRNAs in temperate phage genomes. One hypothesis is that tRNAs are acquired by random chance during **transduction events**; however, no study has found evidence for this hypothesis [20]. Moreover, tRNAs of temperate phages

Box 1. The cellular function of tRNAs

tRNAs make up around 15% of the total ribonucleic acid (RNA) in the bacterial cell, making them the second most abundant RNA after ribosomal RNA (rRNA) [8].

After transcription, tRNAs go through several maturation steps before becoming functional, including the removal of the 5′ and 3′ precursor sequences by a variety of RNases, and the addition of a CCA sequence on its 3′ end by the nucleotidyl transferases (NTases) [9,10]. This CCA sequence is charged with its corresponding amino acid by the aminoacyl-tRNA synthetase (aaRS) based on the anticodon of the tRNA [11–13]. Additionally, tRNAs undergo various post-transcriptional modifications to stabilize the tertiary structure, regulate its translation efficiency, and tune the precise base-pairing of its anticodon [11,12].

After the tRNA is mature and charged with their amino acid, elongation factor thermo-unstable (EF-Tu) guides the tRNA to correct the **aminoacyl site (A site) of the ribosome during translation** [14]. During translation, the amino acid that is attached to the tRNA is incorporated into the nascent polypeptide chain by the ribosome, forming a peptide bond between the amino acids, in accordance with the codon on the mRNA template [12,13,15,16].

To ensure that each of these steps is performed with high accuracy, tRNAs consist of several key features (Figure I) [13]:

- Acceptor stem. This serves as the amino acid attachment site at its conserved CCA sequence at the 3′ end during aminoacylation (the addition of the amino acid to the tRNA).
- Dihydrouridine (D)-arm. This serves to stabilize the L-shaped structure of the tRNA that is required to fit into the ribosome during translation, as well as being crucial for the recognition of the aminoacyl tRNA synthase (aaRS) during aminoacylation.
- Variable loop. This differs in length among different tRNAs, and together with the D-loop, aids the aaRS with distinguishing between tRNAs for the correct aminoacylation.
- TΨC-arm. The assists in stabilizing the L-shaped structure of the tRNA together with the D-loop, as well as aiding the binding of the mature tRNA to the correct position within the ribosome during translation.
- Anticodon loop. Part of the anticodon stem which is responsible for the base pairing of the tRNA with the mRNA codon.

Post-transcriptional modifications can serve a multitude of functions, such as stabilizing the tertiary structure, regulating its translation efficiency, and tuning the base-pairing of its anticodon – for example, through 7-cyano-7-**deazaguanine** and **archaeaosine** modifications of specific tRNA residues [17,18].

Glossary

Aminoacyl site (A site): part of the peptidyl transferase center of the ribosome, where mature tRNAs first enter and bind the ribosome.
Archaeaosine (G^+): a guanine-like nucleobase, also known as 2'-deoxy-7-formamidino-7-deazaguanosine, 2'-deoxy-archaeaosine.
Deazaguanine ($PreQ_0$): a guanine-like nucleobase, also known as 5-Aza-7-deazaguanine or 2-aminoimidazo[1,2-a][1,3,5]triazin-4(1H)-one.
GC content: the percentage of guanine and cytosine in DNA.
Host range: the total number of hosts that the phage can infect.
Hypermodification: modification of a DNA nucleobase at multiple positions.
Infection cycle: also known as replication cycle, this describes how phages replicate within their host, including two main types: lytic and lysogenic cycles.
Lysogenic cycle: a cycle in which the phage integrates into the host genome.
Lytic cycle: a cycle in which the phage replicates and lyses the host cell.
Mobile element: genetic material that can move within or between genomes.
Nonsense suppressor: a mutation in tRNA that leads to the translation process ignoring stop codons and continuing protein synthesis.
Phage satellites: phages that depend on another helper phage to propagate.
RNA interference: RNA that interferes with other RNAs.
Temperate phage: a phage that can alternate between the lytic and lysogenic cycles.
Transduction events: phage-mediated transfer of DNA from one bacterium to another.
tRNA modification: chemical alteration of a ribonucleotide of the tRNA.
tRNA nucleases: also known as tRNases, these are enzymes that cleave tRNAs.
tRNA pool: the set of available tRNAs.
Virulent phage: a phage that exclusively follows the lytic cycle.

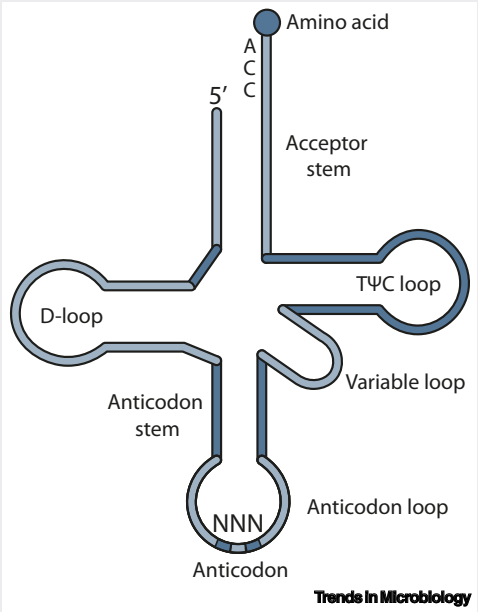

Figure I. Overview of the structural composition of the transfer RNA. Shown are the features of the transfer RNA (tRNA), consisting of the amino acid, acceptor stem, D-loop, anticodon stem, anticodon loop, variable loop and TΨC loop, as well as the CCA-end of the acceptor stem.

The disruption of any of the tRNA maturation steps leads to a variety of detrimental effects, including nonfunctional tRNAs, mischarging, and misincorporation of the tRNA during translation [19]. To resolve these tRNAs from negatively affecting translation, cells have evolved several quality control checks during each of these steps that degrade or repair faulty tRNAs [19].

may function as an integration site for beneficial **mobile elements**, such as those encoding phage defense systems. This benefits the temperate phage in inter-viral conflicts, as observed for *E. coli* P2-like phages and their P4-like **phage satellite**, which encode phage defense systems in a specific integration site in their genome [30]. Lastly, besides possibly providing an advantage in inter-viral conflicts, temperate phage tRNAs may provide an advantage in their conflict with the host defenses. A recent study discovered that temperate Mu phages use a charged $tRNA^{gly}$ from the host as a substrate to hypermodify the adenines of the phage DNA, preventing host DNases from acting [31]. It is plausible that other phages utilize a similar mechanism with their own charged tRNAs.

In addition to regular tRNAs, temperate phages have also been found to encode suppressor tRNAs ($tRNA^{sup}$), also known as **nonsense suppressors** [32–34]. These tRNAs match the stop codon on the mRNA, and instead of terminating the translation process, the tRNA is charged with an amino acid [35]. This incorporation results in readthrough of the stop codon, preventing translation termination, resulting in translation of dysfunctional protein. The role of these suppressor tRNAs in temperate phages has recently been proposed as preventing the correct translation of lytic genes while being in the lysogenic phase, especially in crAss-like phages where the TGA stop codon is re-assigned to glutamine (Figure 1C) [32–34,36].

Phage tRNAs in phage replication and codon usage

While temperate phages encode only a few phage tRNAs, virulent phages can encode up to 61 different tRNAs. Similar to temperate phages, some virulent phages, such as *E. coli* phage T4,

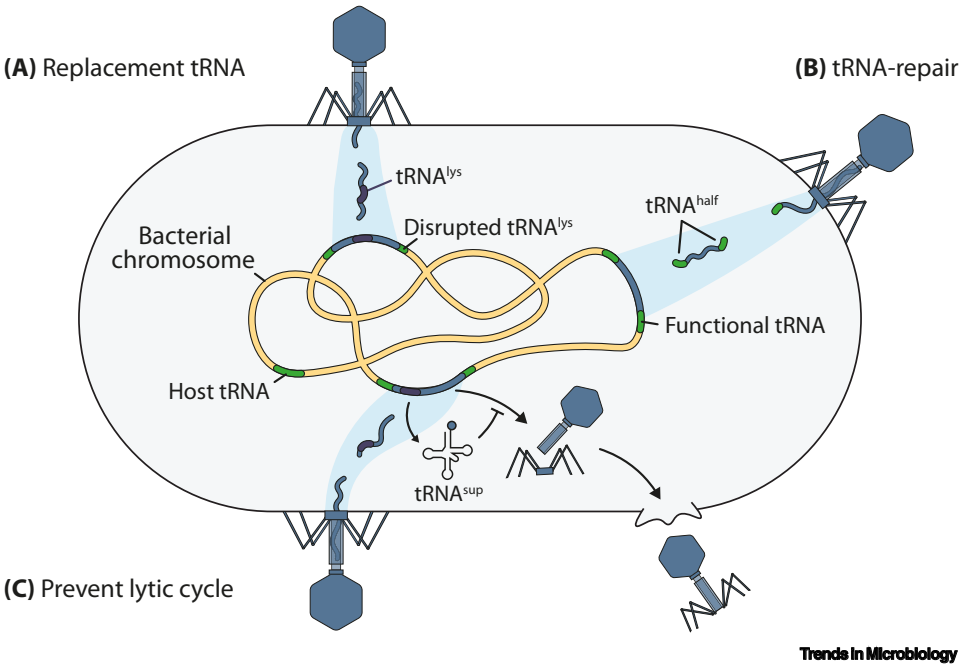

Figure 1. Overview of the tRNA conflict between temperate phages and their hosts. (A) Phage tRNAs are encoded by temperate phages to compensate for the disrupted host tRNA which is caused by the integration of the temperate phage into the host tRNA during its lysogenic cycle. (B) Some temperate phage genomes are flanked by two tRNA halves that restore the function of the disrupted host tRNA after integration. (C) Suppressor tRNAs are encoded to prevent the premature expression of the lytic genes when the temperate phage is integrated into the host genome.

also encode suppressor tRNAs [35,37]. These suppressor tRNAs may function to alter the codon table of the phage, possibly interfering with the translation of the proteins involved in the host response during phage infection [36,38]. Additionally, phage tRNAs of virulent phages may facilitate phage infection through codon compensation, where codons rarely used by the host – but necessary to the phage – are supplemented by the tRNAs encoded by the phage (Figure 2) [20]. Why phages evolved to differ in codon usage from their hosts remains largely unknown. One factor may be that the translation efficiency of certain codons is affected by phage infection, driving the phage to encode more of the efficiently translated codons, while avoiding those that are negatively affected [39,40]. Meanwhile, the host evolved to encode the codons that are well translated during non-phage infection [41]. Another relevant factor for encoding phage tRNAs is to bridge the discrepancies in the **GC content** that are often observed between the phage genome and its host, resulting in a mismatch between the frequently used codons of the host with those of the phage, as well as opening up the **host range** from GC-rich hosts to those with a lower GC content [20,42–45]. This is in line with virulent phages, that do not encode tRNAs, translate their highly expressed genes using the most abundant tRNAs in the host pool, while tRNA-encoding virulent phages use their own tRNAs, especially for their late-stage genes (Figure 2) [20,21,27,46]. This discrepancy may be explained by the **tRNA pool** dynamics during phage infection. Early on in the infection, the host tRNA pool is still abundant enough for efficient translation (Figure 2). However, at a later stage, phage tRNAs take over (Figure 2) [47]. This shift shows the dynamic nature of the tRNA pool during phage infection.

Phage tRNAs replenish the depleted host tRNA pool

The depletion of the host tRNA pool during phage infection was first observed several decades ago [48]. Since then, various causes for this phenomenon have been identified, including the

downregulation of the host tRNAs during the early stages of the infection [49,50] and the active cleavage of the tRNA pool (Figure 3A) requiring phages to evolve countermeasures against these depletion strategies (Figure 3B) [47,50–53]. For example, *E. coli* phage T4 utilizes a tRNA ligase to repair the host lysine tRNAs that are cleaved by PrrC. This cleavage occurs upon detecting the interference by the Stp phage T4 protein of the restriction–modification (RM) system of the host [54].

An alternative and recently identified strategy involves phages encoding tRNAs that are resistant to the host **tRNA nucleases** (tRNases) (Figure 3B) [24,47,55,56]. This strategy was only recently proposed when investigating differences in phage tRNAs compared to host tRNAs [24]. These mutations were found to overlap with known resistance-gaining mutations in the cleavage site of a broad range of tRNA nucleases, including VapC, Colicin E5, Colicin D, and PrrC (Figure 3B) [24]. Several studies have subsequently expanded the relevance of these cleavage-resistant phage tRNAs as an evasion mechanism to other phage defense systems that cleave host tRNAs upon detecting the phage infection, including phage defense systems RM-PrrC, PARIS, Retron-Eco7 (type I-A Retron), Cas13, and RemAIN (Figure 3A) [55–58]. While the impact of phage tRNAs on most of these defense mechanisms is unexplored, their involvement in the evasion of PARIS and Retron-Eco7 is well established [55–58], although their mechanism of action is distinct from each other. Because PARIS (and RM-PrrC) detect the presence of phage proteins that mimic DNA, including Ocr and Stp, both result in the cleavage of $tRNA^{lys}$ [56,59,60], while tRNA-targeting phage defense system Retron-Eco7 is activated by a phage exonuclease that cleaves the multicopy single-stranded DNA (msDNA) of the retron-Septu type II complex, causing the complex to fall apart. This releases the HNH-domain containing effector (PtuB), which starts cleaving the host $tRNA^{tyr}$ or $tRNA^{ser}$, depending on the specificity of the system [55,57].

Moreover, clustered regularly interspaced short palindromic repeats (CRISPR) derived Cas13a complexes, which do not recognize phage proteins. Instead Cas13a recognizes the phage through crRNAs that match phage transcripts with one of the spacers from its CRISPR-array. Once this match occurs, the collateral RNase activity of Cas13a cleaves several host tRNAs including $tRNA^{lys}$, $tRNA^{glu}$, $tRNA^{gln}$, and $tRNA^{thr}$ (Figure 3A) [61,62].

Lastly, RemAIN, a prophage encoded-phage defense system, protects the host from lytic activity of other (pro)phages through cleaving several essential host tRNAs, including $tRNA^{leu}$, $tRNA^{pro}$, $tRNA^{fmet}$, and $tRNA^{ser}$, upon detecting an unknown trigger (Figure 3A) [58].

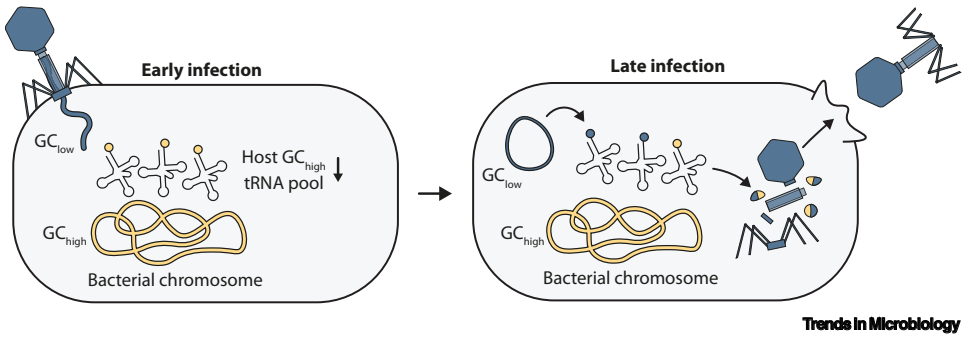

Figure 2. Phage tRNAs in phage replication and codon usage. Phage tRNAs may compensate for the differences in GC content between the phage and host. Early phage translated genes are translated using the most abundant host tRNAs. However, at later stages, phage genes are translated using the tRNAs of the phage.

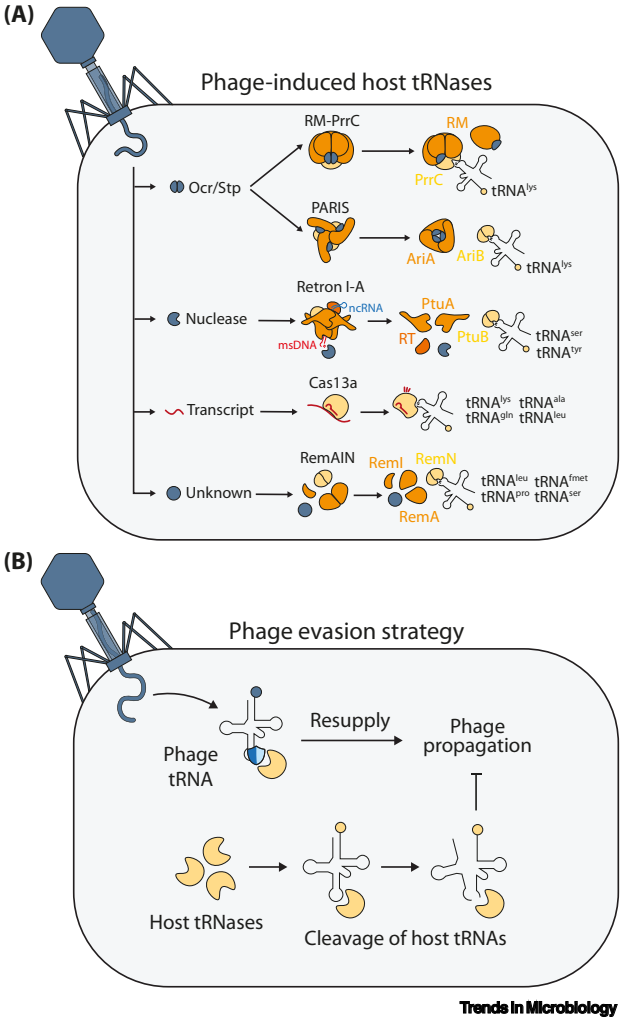

Figure 3. Phage tRNAs replenish the depleted host tRNAs. (A) During phage infection, several phage factors are known to activate the tRNases of the host, such as DNA mimics Stp and Ocr, which interfere with the defense response of the host, and induce the activity of RM-PrrC and PARIS to cleave the $tRNA^{lys}$ of the host. In addition, Retron-Eco7 (type I-A Retron) is activated by an exonuclease of the phage, resulting in the cleavage of the msDNA of the retron. This causes the Retron-Eco7 complex to disassemble and start cleaving the $tRNA^{tyr}$ or $tRNA^{ser}$ of the host. Moreover, Cas13a is activated by phage transcripts that match its spacer, leading to the cleavage of several host tRNAs, including $tRNA^{lys}$, $tRNA^{gln}$, $tRNA^{ala}$, and $tRNA^{leu}$. Lastly, RemAIN, a prophage encoded-phage defense system, protects the host from lytic activity of other (pro) phages through cleaving several essential host tRNAs, including $tRNA^{leu}$, $tRNA^{pro}$, $tRNA^{fmet}$, and $tRNA^{ser}$, upon detecting an unknown trigger. (B) The cleavage of tRNAs by these phage defenses results in the inhibition of translation and phage propagation. In response, phages encode tRNAs, that are resistant to these host tRNases to replenish the tRNA pool and propagate.

Although the involvement of phage tRNAs in evading each of these phage defense systems has not yet been demonstrated, current evidence strongly suggests their potential to neutralize these phage defenses. It is also likely that the number of tRNA-targeting defense systems exceeds the abovementioned defenses, since the functional protein domain that cleaves tRNAs for each of these phage defense systems is shared across various phage defense systems. PARIS contains the Old/TOPRIM functional domain, which is responsible for their tRNA cleaving capacity. This domain is also present in at least 11 other phage defense systems, including Menshen and Gabija [63–70]. Moreover, Retron-Eco7 (type I-A retron) cleaves tRNAs using its HNH nuclease domain, a commonly shared domain among phage defense systems, including Septu type I, Zorya type II, RM type II systems, and CRISPR-Cas systems [63,71,72]. Additionally, Cas13a, RemAIN, and PrrC share their tRNA cleaving HEPN domain with at least 17 other phage defenses, including CoCoNuTs, AbiF, and Ape [65,68,70,73–79]. Lastly, VapC shares its PIN domain with phage defenses HEC-03 and HEC-09 [68,80]. While these domains are not necessarily always involved in tRNA cleavage, and may have different nucleic acid targets, it is likely that at least some of these phage defenses target tRNAs, potentially even the tRNAs of the phage. Promising candidates may be found in the VapC family, as many VapCs have unknown tRNA targets, while extensively investigated for their activity against the tRNAs of the host [81].

Proposed phage tRNA functions

In addition to the abovementioned roles and proposed hypotheses regarding the role of tRNAs in phages, several additional novel hypotheses may be proposed, one of which is that some phage tRNAs are not resistant to cleavage and instead act as tRNA-derived small RNAs (tsRNAs) and tRNA-derived fragments (tRFs), which are known to modulate the transcription and translation of the host [82]. Moreover, phage tRNAs might act to stall the ribosomes of the host during infection, preventing a host defense response [83], or interfere with other tRNA-binding proteins [84]. Alternatively, integrated temperate phages might encode tRNAs to increase the available tRNA pool of the host, which may accelerate their replication, resulting in an increased number of integrated phage copies. Lastly, eukaryotic viruses, such as human cytomegalovirus, have been found to use tRNAs as scaffolds to assemble their capsids. It is plausible that phages use tRNAs in a similar fashion [85].

Taken together, phage tRNAs have diverse functions. In temperate phages, tRNAs may restore the detrimental effects of their integration into the tRNA of the host and prevent the premature expression of their lytic genes. In virulent phages, tRNAs are primarily encoded to benefit the phage during its replication cycle, to counteract the phage defense response of the host and overcome discrepancies in codon usage between phage and host genes. Notably, these lytic-related functions could also apply to the functionality of the tRNAs of temperate phages that have entered the lytic cycle. More speculative functions of phage tRNAs include their involvement in the **hypermodification** of phage DNA to provide an integration site for beneficial mobile elements, increase the tRNA pool of the host for improved replication, function as capsid scaffolds, and regulate host translation and transcription through **RNA interference** and ribosomal stalling.

Layers of tRNA-targeted phage defense

Cleavage of host tRNAs is not the only tRNA-targeted layer of phage defense that the host immune response initiates upon phage infection (Figure 4). Another significant mechanism involves the toxic small alarmone synthetase (toxSAS) family, including toxSAS and phage defense system CapRel, which pyrophosphorylate the highly conserved CCA-ends in the acceptor stem of tRNAs to inhibit translation and prevent phage propagation (Figure 4B) [86,87]. Other host mechanisms that act on tRNAs, though not yet linked to phage defense, are part of the nucleotidyltransferase (NTase) family [88]. The most notable member of the NTase family is the MenT toxin of *Mycobacterium tuberculosis* [88]. MenT extends the tRNA at the acceptor stem with additional cytosines, preventing the aminoacylation process during maturation and inhibiting translation (Figure 4B) [88]. Other members of the NTase family are less understood but are known to convey phage defense, including SanaTA, AbiE, and AbiG [67,89]. Lastly, TacT, a member of the acetyltransferase (GNAT) family, disrupts translation through acetylation of the glycine aminoacyl-tRNAs (Figure 4B).

In addition to these toxins, it is conceivable that hosts distinguish self-tRNAs from non-self-tRNAs through several specific modifications to the tRNA during maturation [13]. To bypass these host defenses, phages may act to become independent of the host and encode their own tRNA-maturing enzymes to steer the phage tRNA pool away from these host regulatory pathways (Figure 4A) [90]. Supporting this hypothesis, a recent study has found that some phages encode not only one, but several steps of tRNA maturation, including their own CCA-adding tRNA NTases, aminoacyl-tRNA synthetases (aaRS), and elongation factors (EF-Tu), as well as tRNA-modifying enzymes such as QueC and tRNA-guanine transglycosylase (TGT)-like proteins [17,18,90], which are known to modify the guanine (G) of tRNAs into 7-cyano-7-deazaguanine ($preQ_0$) and archaeaosine (G^+) through their involvement in the deazaguanine metabolism (Figure 4A) [17,18]. The exact benefit for phages to modify the guanine (G) into $preQ_0$ or G^+ remains to be speculated. A similar **tRNA modification**, deazaguanine derivative queuosine (Q), is present at the first position of the anticodon and

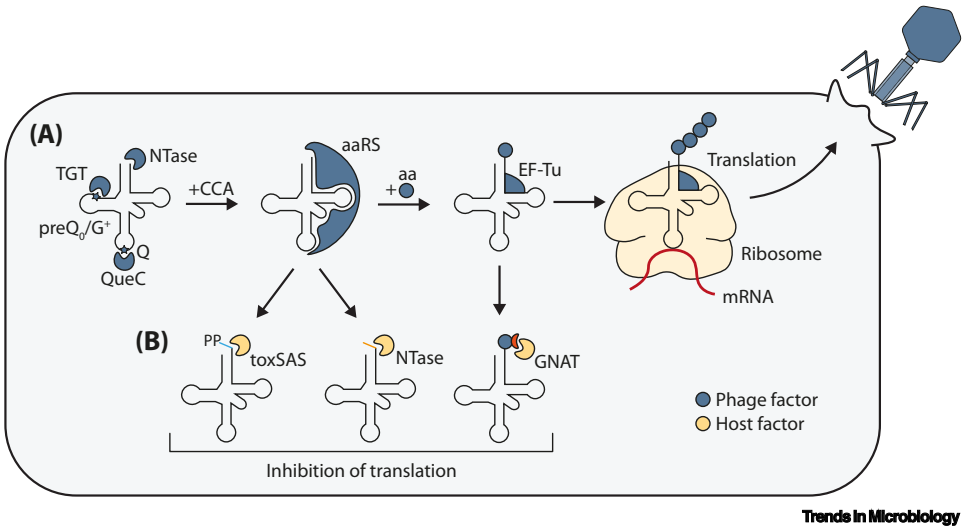

Figure 4. The layers of tRNA-targeted phage defense in the maturation steps of phage tRNA. (A) The stages of phage tRNA maturation are shown, from transcription to tRNA modifications, processing, amino-acetylation, translocation, and translation. Phage factors are shown in blue, while bacterial factors are shown in yellow. (B) The proposed host defenses that may interfere with the maturation of the phage tRNA are also illustrated, including toxSAS, NTase, and GNAT. Abbreviations: aa, amino acid; aaRS, aminoacyl-tRNA synthetase; EF-Tu, elongation factor thermo-unstable; G^+, archaeaosine; GNAT, Gcn5-related N-acetyltransferases; NTase, nucleotidyltransferase; preQ0, 7-cyano-7-deazaguanine; Q, queuosine; TGT, tRNA-guanine transglycosylase; toxSAS, toxic small alarmone synthetase.

improves the codon-anticodon interaction, making the translation faster and more accurate [91,92]. However, unlike Q, $preQ_0$ and G^+ modifications are present in the D-loop and act to improve the stability of the tRNA during stressful conditions, which might be beneficial for the phage during phage infection [93]. Moreover, $preQ_0$ and G^+ may render phage tRNAs more resistant to the tRNA cleaving enzymes of the host, since the G^+ modification in DNA is known to render DNA resistant to a multitude of endonucleases, including EcoRV, HaeIII, DraI, EcoRI, and MboI [94]. In addition, $preQ_0$ or G^+ modification might also be a way to distinguish phage tRNAs from host tRNAs [13].

These complex dynamics highlight the arms race between the host and phage, with numerous unresolved questions to be explored.

Concluding remarks and future perspectives

Although phage tRNAs have intrigued scientists since their discovery in the 1960s, it was only recently that researchers have begun to understand their complexity, uncovering an array of unexpected roles for phage tRNAs during phage infection. These diverse roles make predicting the function of individual phage tRNAs challenging and are likely affected by multiple factors. However, certain overarching functions appear to be conserved, such as virulent phages are known to encode a multitude of tRNAs to overcome the defenses of the host and improve translation efficiency. For temperate phages, tRNAs are likely encoded to prevent a detrimental effect of integrating into the tRNA of the host, as well as controlling the host response during the lytic and lysogenic stages. These novel insights provide promising potential for phage-therapeutic approaches. For instance, natural or genetically engineered phages, that encode additional phage tRNAs, could significantly enhance the efficiency of the phage infection to overcome phage resistant bacteria that utilize tRNA-depleting defense strategies. Furthermore, engineering phages might also be a way to expand their host range by overcoming codon usage discrepancies

Outstanding questions

How common are phage defense systems that cleave host tRNAs?

Are there phage defense systems that specifically target phage tRNAs?

Is there a signaling role for tRNA fragments during phage infection?

Are phage tRNAs encoded by phages as mobile element integration hotspots?

Can we extrapolate the roles of phage tRNAs to tRNAs encoded by eukaryotic viruses?

between the host and phage. A comprehensive understanding of phage tRNAs will be crucial for these applications. Intriguingly, several unexplored hypotheses remain that may be validated in the years to come (see Outstanding questions). These questions might be answered with a recent burst of novel methods to investigate tRNAs *in vivo*, such as the improved detection of tRNA modifications and high-throughput sequencing of tRNAs [95–97]. Understanding the full complexity and role of phage tRNAs holds the potential to benefit phage-based therapeutics through empowering phages to overcome tRNA-targeting phage defenses [98].

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Declaration of interests

No interests are declared.

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